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ORIGINAL PAPER

Persistent and advanced reddening of sweetgum leaves after major veins severing

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Abstract: The effects of major veins severing on morphological and physiological features of sweetgum (*Liquidambar styraciflua* L.) leaves were investigated by observing leaf color change and measuring leaf temperature, green/luminance (G/L) value of half-lobes, leaf stomata conductance, and water content in Yamaguchi University, Japan. The palmately veined leaves of sweetgum (*Liquidambar styraciflua* L.) were found more sensitive to the major vein severing than that of other species. Major veins severing resulted in serious water stresses, as indicated by the persistent reddening and/or advanced reddening of local leaf, lower leaf stomatal conductance, and higher leaf temperature, etc. Severed leaf can be clearly divided into non-severed area, transitional area, and stressed area, which the three areas have different colours and temperature. The major vein barrier can also be seen clearly. The persistent reddening and advanced reddening seem consistent with the phenomenon of red crown top of some sweetgum trees and may have similar mechanism.

Keywords: advanced reddening; G/L value; leaf vein severing; persistent reddening; red top crown; stomatal conductance; sweetgum; water stress

Introduction

Morphologies of plants or trees are usually the equilibrium between genetic property and environmental effects. The catastrophically environmental extremes occasionally happen in field, it can generate direct damage to plants (Baig and Tranquillini 1980; Chiba 1994; Yamamoto et al. 1996), also induce plant protective responses (Alexieva et al. 2001; Liu et al. 2007). Some tree species or individuals at special stages or status are especially sensitive to environmental extremes such as excessive irradiation. Excessive irradiation energy is a relative concept (Fitter and Hay 2002). For stressed plants or trees, even the scatter light might be occasionally excessive to them. Plants or trees usually protect against excessive photo energy acceptation through heat transformation (Donald 2001; Fitter and Hay 2002) and anthocyanin accumulation in leaves (Chalker-Scott 1999). Leaf transpiration is an important procedure of excessive heat energy dissipation (Clements 1934; Gates 1968; Fitter and Hay 2002). Stomatal status plays an important role in leaf transpiration. Anthocynin content in a leaf is a negative regulation factor of leaf stomatal conductance (Farooq et al. 2009), thus anthocyanin accumulation might induce protective response to the excessive light or heat energy.

Many plant species with leaf major vein severing were reported to survive without apparent symptom (Wylie 1927; 1930). Conversely it is also reported that the major vein severing can induce leaf death or dysfunction of water transportation in leaf lamina for some tree species (Sack et al. 2003). Expanding leaves on new shoots of many tree species appeared red or non-green colors, which are often called “delayed greening” (Lambers et al. 1998; Numata et al. 2004). The expanding or fully expanded sweetgum (*Liquidambar styraciflua* L.) leaves with the characteristic of delayed greening often appeared persistent red or purplish red color at stressed part after severing the major veins. It is named as the “persistent reddening” in this paper. In contrast, the chlorophyll mature leaves early becoming red or purplish red at stressed part after severing the major veins is called “advanced reddening” in the study. The appearance of red top crown, persistent reddening and advanced reddening of sweetgum leaves seems to be an instance of transpiration cooling failure. In some extent, an increase in leaf anthocyanin content is considered as the results of inter-relation of many environmental factors such as photoperiod and drought stress (Alexieva et al. 2001; Yang et al. 2005; Farooq et al. 2009). According to the measurement of leaf stomatal conductance, thermo image temperature, water content measurement and RGB image analysis, the present study inferred that the sweetgum leaves with major veins severed may be one of the examples special sensitive to the excessive irradiation energy and water stress.

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Materials and methods

The study was conducted in Yamaguchi University, Japan. The observed sweetgum trees were several new sprouting plants from stem cutting stocks, one old stem sprouting plant, and some street trees in Yamaguchi City, Japan. During the study, leaf laminas of sweetgum usually were divided into 10 half-lobes in responding to their special palmate venation and in the order against hour hand. If there are seven lobes on the leaf, the smallest two lobes at leaf base are incorporated into half-lobe 1 or 10. Analysis of RGB images, thermo images and water content of leaf laminas were conducted by using these half-lobes as basic unit.

Leaf temperature was determined by using thermography (Jones 1999; Jones et al. 2002; Jones and Leinonen 2003; Prytz et al. 2003). In this study, a NEC TH7100 thermal infrared (8–14 µm) camera, with the temperature measuring range from –20°C to 100°C and minimum sensible temperature 0.06°C, was hand-held about 50 cm above the objective leaves and then focused to clear. Thermo image temperature was measured by using the active heat method in field under direct sunshine heating from 8:00 to 10:00 a.m. Smoothly expanded leaves were selected to take the thermo images.

Water content of the leaf half-lobes, directly cut from attached leaves and taken back to lab with plastic bags, was measured by rapid weighing method in room temperature with an electronic balance (Shimadzu Auw220, 1/10000 g) on Sep. 21, 2009. The water content of non-severed area (N), transitional area (T) and stressed area (S) was the average value of half-lobes in these areas.

The Green/Luminance (G/L) value of half-lobes is a proportion between green and luminance value from RGB images. It was obtained with image analysis method (Wang et al. 2008, 2009a, 2009b) to describe the persistent reddening and advanced reddening of leaves. Images were scanned with a scanner (Canon D125u2) or taken with a CCD camera (Canon IXY 6.0). The photo making (PM) and vein severing (VS) date were noted at figure caption, respectively.

Leaf stomata conductance from different areas on the same leaf was measured with a SC-1 leaf porometer in clear field environment from 9:00 to 11:00 a.m. on Sep. 10, 2009. During the measurement, the sensor clip was fixed on non-severed, transitional, and stressed half-lobes of attached leaves and automatically measured.

Results and discussion

By observations, juvenile leaves from sweetgum showed varied coloration and were green or light green during leaf expansion in early April in Yamaguchi. However, some of sweetgum trees in growing season presented delayed greening leaves on top of the crown or twigs, especially the trees growing at constricted sites and/or during the extreme hot/dry period (Fig. 1a). As the leaves grew, the delayed greening leaves became green gradually and usually maintained two apical red leaves on shoots as reported by Hughes et al. (2007). The persistent reddening of expanding leaves locally appeared on leaves by carefully designed experiment in this study. Sweetgum leaves usually possess five or seven lobes on a leaf lamina with one main vein on each lobe (Fig. 1a). Their major-veins and sub-major-veins terminate at the end of leaf edge. There is no anastomose among major veins or sub-major veins, and only local minor veins anastomose at the joint part between two lobes (Fig. 1b). If the major vein in one lobe is severed, the leaf lamina of the lobe would have to obtain water only from the nearest non-severed lobes via the minor vein joints. If more than one adjacent major vein is severed, there is always a part far from theses minor vein joints. Therefore, it is easy to induce the local leaf lamina far from these joints into water imbalance by major vein basally severing, for instance, the II, III and IV major veins severing (Fig. 1a, 1b), especially for the rapid expanding younger leaves. As the stress continues, persistent reddening occurred (Fig. 1b-S, 1c-S) at the local area. Severed leaf can be clearly divided into non-severed area (Fig. 1b-N, 1c-N), transitional area (Fig. 1b-T, 1c-T), and stressed area (Fig. 1b-S, 1c-S).

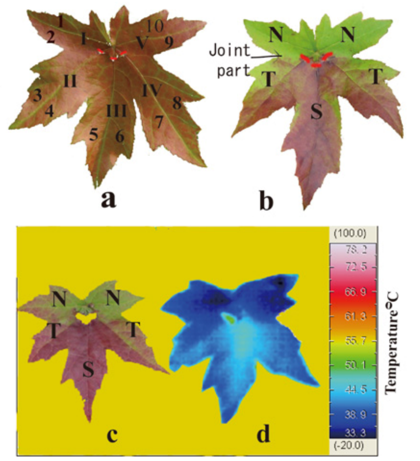

Fig. 1 A persistent reddening juvenile sweetgum leaf with five major veins signed with I, II, III, IV and V, respectively. (a, photo making (PM) and vein severing (VS) on Jun. 1). The leaf is severed at the base of II, III and IV major veins with mark "—"and divided into 10 half-lobes in the order against hour hand. The same leaf (b, PM on Aug. 1) with the persistent reddening area at stressed part (S, half-lobes of 4,5,6 and 7), with greened area at non-severed part (N, half lobs of 1,2,9 and 10) and the transitional area between them (T, half-lobes of 3 and 8); another persistent reddening stem-developed juvenile leaf (c, VS on Jun. 1 and PM on Aug. 1) and its thermograph (d, PM on Aug. 18). The persistent reddening test had been duplicated more than six leaves and these two are typical examples.

The non-severed area usually turned to green color as the leaf grew, while the stressed area maintained red color or purplish red color. The transitional area showed the intermediate coloration and more greening near the minor vein joint (Fig. 1b). The persistent reddening even persisted in all of the growing season. It was observed that the major veins of II and IV became the demarcation line between persistent reddening area and greened area (Fig. 1b, 1c) for the leaves of II, III and IV major veins severed. This means they are the main barriers to interrupt water transporting into the half-lobes next to the severed veins. Therefore, they are important lines dividing the stressed area, transi-

tional area and non-severed area. By thermograph taking, a high temperature area was observed at the farthest part from non-severed-area (Fig. 1d). From the thermo-image, the major vein barrier and the difference among non-severed area, transitional area and stressed area were also seen clearly. However, this difference was not observed on the normal leaves. It implies significant difference of leaf temperature before and after leaf severing.

Using the same major vein severing method, partially advanced reddening on chlorophyll mature leaves have also been induced (Fig. 2). It is clear that the advanced reddening from severed leaf (Fig. 2b) appeared significantly different color from its normal status before severing (Fig. 2a). While it was similar to that of severed leaves in persistent reddening process (Fig. 1b, 1c), which showed red color in the stressed area. The major vein barrier from vein II and IV also appeared on the leaves of II, III and IV major vein severed in the advanced reddening process. In the situation of major veins III and IV severed, the major vein barriers also could be seen and showed slight leaf advanced reddening (Fig. 2c-S) at stressed area. In fact, the difference of leaf stomata conductance (Fig. 2c-L) and G/L value (Fig. 2c-G) from RGB images have been measured among the non-severed area (Fig. 2c-N), transitional area (Fig. 2c-T) and stressed area (Fig. 2c-S). The leaf similarly showed the high temperature area (Fig. 2d-S), transitional temperature area (Fig. 2d-T) and low-temperature area (Fig. 2d-N) as well as the major vein barrier from veins III and IV 18 min after III and IV major veins severed. It is clear that the high temperature and low leaf stomata conductance (Fig. 2c-L) occurred in stressed area, which was caused by the termination of direct water supply (Fig. 2b-w). This tendency not only appeared on sunlit leaves but also occurred on shaded leaves (Fig. 2e, 2f). It also showed partially advanced reddening in stressed area on RGB image (Fig. 2e) and high temperature area in thermo-image (Fig. 2f), although the relative area was smaller than that appeared on the leaves shown in Fig. 2b and it took a longer period for the symptom occurrence. Both the persistent reddening and advanced reddening of the major veins severed leaves were tree specific or age specific. Fig. 2g presented an entirely reddening leaf on the main stem of a sensitive tree to abiotic or biotic stimulation, whose leaves are usually small, thin and light colored. The phenomenon of major vein barrier could still be seen on this leaf. On the other hand, a recently pruned tree with large, thick and deep green colored leaves maintained greening (Fig. 2h). By measurement, the difference of water content among the non-severed, transitional and stressed area was very small even no statistically meaningful difference. It matched with the phenomenon that leaves on pruned trees often turn yellow but not red or purplish red in the late autumn.

Therefore, acute reduction of water supply after major-vein severed, especially for multi-vein-severing, caused the leaf stomata close as indicated by the immediate reduction of leaf stomata conductance. The relative high temperature on stressed area of leaf lamina should be the direct result of transpiration cooling failure caused by stomata closure and the water content reduction. The evident temperature limit from major vein indicates that inversely transport water into the area far from water source is difficult. This kind of barrier properly matches the limit line between persistent reddening area and greened area of severed sweetgum leaves. It indirectly suggests that there is a potential to pre-detect the water stress, persist-reddening and pre-reddening status of these sweetgum leaves by using thermography as previous researches (Chaerle and Van Der Straeten 2000; Grant et al. 2006). The persistent reddening and advanced reddening of sweetgum leaves occurred in the process of persistent water, light and heat energy imbalance after leaf severing. The photoprotection mechanism in the sweetgum leaves triggers the anthocyanin production and causes the leaf persistent reddening and advanced reddening.

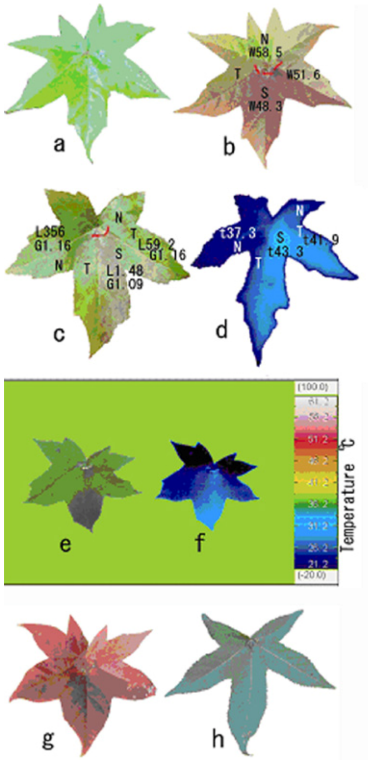

Fig. 2 A normal chlorophyll mature leaf (a, PM and VS on Aug. 9) and the stressed area advanced reddening for the same leaf after II, III and IV major veins severed (b, PM on Sep. 21) with the severed position marked with "—" and water content (w) at non-severed area (N), transitional area (T) and stressed area (S); A slightly advanced reddening leaf at the stressed area (c-S) after III and IV major veins severed (c, VS on Jul. 6 and PM on Sep.6), in which the number next to "L" is the leaf stomata conductance value and next to "G" is the ratio between green and luminance values from RGB image; The thermograph (d, VS on Aug.11and PM 18 minute after VS) of a III and IV major veins severed leaf with different image temperature (t); The thermograph (f, PM on Aug.18) and the RGB image (e, VS on Jun.1 and PM on Aug.18) of a shade leaf; an entire red leaf (g, VS on Aug. 9 and PM on Sep.21) with advanced reddening area and an entire green leaf (h, VS on Jul. 5 and PM on Sep. 4) without advanced reddening area. Leaves in e, f, g and h are II, III and IV major veins severed. The advanced reddening test had been duplicated more than twenty times and here are some typical examples.

Significantly changed characteristics of climate appeared in Yamaguchi, Japan from 2006 to 2008 accompanying with some extreme weather events, such as extreme strong wind mingled with less rainfall during hit by typhoon number 13 in 2006 (T0613) and persistent high temperature and drought in 2007 (Wang et al. 2009b). Prolonged vegetative growth of sweetgum trees benefited from the abundant precipitation during the growing season in 2006 and almost showed no red top crown (Fig. 3a),

although the T0613, characterized by strong wind and less rain accompanying with more than one month of no rain period, made the crown of sweetgum trees asymmetrically leaf scorching from windward to leeward. During 2007 and 2008, the persistent extreme weather of high temperature and less rainfall, especially during the growing season (Fig. 3b), induced the sweetgum trees into asymmetrically advanced discoloration from top to base of their crowns (Fig. 3a) in fall. By integration, the red top crown phenomenon was consistent with the precipitation during the growing season (Fig. 3b). It also indicates that the heavy rainfall in 2006 provided sufficient water supply to soil system, met the normal transpiration cooler requirement of the trees and reduced the impact from summer heat weave. The persistent reddening and advanced reddening seem consistent with the phenomenon of red crown top of some sweetgum trees and may have similar mechanism.

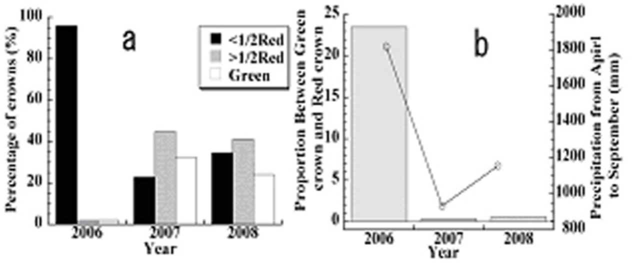

Fig. 3 The observation results of red crown top of sweetgum trees in mid-October in 2006, 2007 and 2008 (a), in which single sweet gum trees along the high way or street in Yamaguchi were visually scaled into classes of entire crown green (Green), reddening crown <1/2 (<1/2 Red) and reddening crown >1/2 (>1/2 Red); the proportion between green crowns and reddening crowns (b, histogram), and the precipitation from April to September (b, ○─○). The precipitation data was obtained from Automated Meteorological Data Acquisition System of Japan.

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